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Anti-smoking legislation and its effects on urinary cotinine and cadmium levels

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ABSTRACT

Anti-smoking legislation has been associated with an improvement in health indicators. Since the cadmium (Cd) body burden in the general population is markedly increased by smoke exposure, we analyzed the impact of the more restrictive legislation that came into force in Spain in 2011 by measuring Cd and cotinine in first morning urine samples from 83 adults in Madrid (Spain) before (2010) and after (2011) introduction of this law. Individual pair-wise comparisons showed a reduction of creatinine corrected Cotinine and Cd levels for non-active smokers, i. e. those which urinary cotinine levels are below 50 μg/L. After the application of the stricter law, cotinine levels in urine only decreased in non-active smokers who self-reported not to be exposed to second-hand smoke. The reduction in second hand smoke exposure was significantly higher in weekends (Friday to Sunday) than in working days (Monday to Thursday). The decrease in U-Cd was highly significant in non-active smokers and, in general, correlated with lower creatinine excretion. Therefore correction by creatinine could bias urinary Cd results, at least for cotinine levels higher than 500 μg/L.

The biochemical/toxicological benefits detected herein support the stricter application of anti-smoking legislation and emphasize the need to raise the awareness of the population as regards exposure at home.

1. Introduction

Tobacco use contributes to respiratory, renal and cardiovascular problems, increases the risk of cancer, and is considered to be the single most significant cause of preventable diseases, disability and premature mortality in most developed countries (CDC, 2013).

The implementation of comprehensive national anti-smoking legislation (banning smoking in indoor public spaces or in all workplaces) has been associated with improvements in indoor air quality and in a number of health outcomes in non-smoking workers and the general population, including fewer respiratory and sensory symptoms and improvements in lung function (Hahn, 2010; CDC, 2013). Moreover, such legislation has been associated with reductions in the overall number of heart attacks and asthma-related hospital admissions (Institute of Medicine, 2009; Sims et al., 2013), and of preterm births (Kabir et al., 2009).

Spain became a party to the WHO Framework Convention on Tobacco Control in 2005. The first legislation governing smoking in public places and tobacco advertising, promotion and sponsorship (Law 28/2005) achieved a considerable reduction in exposure to environmental tobacco smoke in the workplace and, to a lesser extent, in bars and restaurants (Galán et al. 2007). In January 2011, new legislation (Law 42/2010) substantially amended the previous law by mandating an additional ban on smoking in indoor public places and workplaces, and repealed provisions that permitted designated smoking rooms, which had been used to continue to allow smoking in most bars, restaurants and airports. According to Spain's National Statistics Institute, in 2001 31.7% of the population

Abbreviations: ; SHS, second-hand smoke; SHSe, SHS exposure; U-Cd_{cr}, urinary creatinine-corrected cadmium; U-cot_{cr}, urinary creatinine-corrected cotinine; ICC, the intra-class correlation coefficient; BMI, body mass index

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smoked daily, whereas this prevalence dropped to 24.0% in 2012, the lowest for the past 25 years (INE, 2013).

Cigarette smoke contains more than 7000 chemical substances, including nicotine, cotinine and toxic metals such as cadmium (Cd) (CDC, 2013). Cotinine is also the major metabolite of nicotine, with a longer half-life, and, as such, is frequently used to distinguish smokers from non-smokers and in studies of passive smoking (O′ Connor et al., 1995). For the general population, cigarette smoking may represent a major source of chronic exposure to Cd. The amount of this metal absorbed upon smoking one pack of cigarettes per day is about 1–3 μg/day (ATSDR, 2012). Smoking roughly doubles the Cd body burden in comparison to not smoking, mainly in the kidney, as reflected by urinary Cd (U-Cd) (ATSDR, 2012). Furthermore, a very large fraction of Cd is released into side-stream smoke, which is the main component of second-hand smoke (SHS) (Wu et al. 1995). Passive smoking has been related to increased Cd levels in blood (Shaham et al., 1996) and children's hair (Razi et al., 2012), although other authors have found no such association (Becker et al., 2007; McElroy et al., 2007; Richter et al., 2009).

Both Cd and tobacco smoke are classified by the International Agency for Research on Cancer as carcinogenic for humans, Group 1 (IARC, 2013). Long-term human exposure to Cd has been associated with renal, cardiovascular, lung and bone diseases (Järup and Akesson, 2009; ATSDR, 2012), reproductive, fetal-growth and child-development problems (Satarug and Moore 2004; Kippler et al. 2012; Ciesielski et al. 2012) and other diseases (Han et al., 2009; Satarug et al., 2010). Indeed, Cd may mediate the effect of smoking on the development of tobacco-related lung disease (Mannino et al., 2004; Lampe et al., 2008) and peripheral artery disease (Navas-Acien et al., 2004).

In light of the above, it is important to establish whether a stricter smoking ban has an impact on the reduction of cotinine and Cd load in the general population. To this end, we have evaluated whether urinary cotinine and Cd excretion in a group of adults living in Madrid (Spain) was modified after application of the above-mentioned law.

2. Material and methods

2.1. Recruitment

During their annual medical check-up, workers from the Instituto de Salud Carlos III (ISCIII) in Madrid (Spain) were asked to participate in a biomonitoring study running from 2007 to 2011 (Castaño et al., 2012). Each year, participants were requested to sign an informed consent, complete a self-administered questionnaire and provide a first morning urine sample. The questionnaire included lifestyle habits, such as smoking and smoke-exposure patterns. Verification of the subjects' smoking habits and body mass index (BMI) was obtained from the occupational health physician's report. Both the questionnaire and the study were approved by the ISCIII's Prevention Service to comply with the Declaration of Helsinki for human subject research (WMA, 2008).

A total of 83 non-occupationally exposed participants (59 women and 24 men) aged 25–62 years, whose urine samples were collected in both 2010 and 2011 and had creatinine values of between 0.3 and 3 g/L, were selected for inclusion in this study. This cohort constitutes a very stable population with no great variations in lifestyle habits and diet, as seen from the questionnaires and a preliminary comparison of the main dietary food groups (vegetables, pasta, bread/cereals, legumes; data not shown). Ferritin serum levels were relatively constant for each participant in the study period and higher than 6.5 μg/L for women and 16.6 μg/L for men.

2.2. Chemical analysis

Urine samples were aliquoted and stored at −20 °C in polypropylene tubes prewashed with 10% HNO_3 until analysis. Creatinine concentrations were determined for 1:40 dilutions using the Jaffé alkaline picrate method (Spinreact Kit, Spain).

Urinary cotinine (U-cot) was first determined by HPLC using a Diode Array Detector (Bartolomé et al., 2014). Briefly, 5 ml urine plus 2-phenylimidazol, as internal standard, was extracted on a solid phase using Oasis HLB cartridges (Waters, USA), evaporated to dryness under nitrogen and reconstituted with mobile phase solution (milli-Q water, acetonitrile). A 25 μL aliquot was injected into an HPLC system (Agilent Technologies, 1200), with detection at 259 nm (LOQ 15 μg/L).

For U-cot concentrations below 25 μg/L, the urine extract was reconstituted with 50 μL toluene and a 2 μL aliquot was injected into a single quadrupole, selected ion monitoring, electron impact gas chromatography–mass spectrometer (Agilent Technologies 5975C), with an LOQ of 1 μg/L. The results are expressed as creatinine-corrected cotinine (U-cot_{cr}).

Urinary cadmium (U-Cd) analyzes were performed in the same run, from October to December 2012, using a Dynamic Reaction Cell (DRC)-ICP-MS (PerkinElmer ELAN DRC-e), as described by Cañas et al. (2013). Briefly, mixed urine samples were diluted 1:5 in 10% HNO_3 containing 20 μg/L rhodium as internal standard (Merck KgaA, Germany) and the DRC parameters were a flow rate of 1.7 mL/min oxygen (Air Liquide) and 0.7 Rpq. External calibration ranges were 0.025–10 μg/L (Cd standard, Perkin Elmer, USA) and the limit of quantification (LOQ) was 0.025 μg/L. As an internal quality control check, the accuracy of the results was checked by analysis of the certified reference material Clincheck® urine (Recipe, Germany) analyzed every ten samples. The values obtained (2.33 ± 0.19 μg/L, $N=17$, and 1.81 ± 0.06 μg/L, $N=16$) were in good agreement with the certified values (1.94–2.90 μg/L and 1.71–2.57 μg/L, respectively).

During the measurements we participated in three Quebec Multielement External Quality Assessment Schemes (QMEQAS) as an external quality control. The values obtained (0.84 ± 0.06, 2.29 ± 0.07 and 14.03 ± 0.13 μg/L) were in good agreement with the consensus values (1.07 ± 0.15, 2.50 ± 0.16 and 14.06 ± 0.90 μg/L, respectively). The coefficient of analytical variation for U-Cd was 10%.

2.3. Statistical analysis

All analyzes were performed using PAWS Statistics 19 and STATA 11. Urine levels below the LOQ were replaced with LOQ/$\sqrt{2}$ (Hornung and Reed, 1990). Two groups were established for active / passive smoking behavior, thus, in the active smokers group were included those individuals with U-cot levels above or equal 50 μg/L (SRNT, 2002). The urinary biomarker levels estimated before and after introduction of the stricter anti-smoking legislation, i.e. in 2010 and 2011, were compared using Wilcoxon test for related samples. Comparisons between groups of different U-cot level or SHSe in non-active smokers were performed using Mann Whitney *U*-test for biomarker concentrations and a *z*-test for proportions. Statistical significance was established at $p < 0.05$.

The intra-class correlation coefficient (ICC), i.e. the inter-participant variance divided by the sum of inter- and intra-participant variance, was used to compare the agreement between repeated measurements of continuous variables. Additionally, Spearman's or Pearson's correlations were used to estimate associations between concentration measures and between the analytes quantified.

3. Results

Table 1 outlines the age, gender distribution, BMI and smoking habit (urinary cotinine groups) for the study population. No statistically significant differences in terms of either gender composition (about 71% women and 29% men) or age were found between U-cot groups (passive- and active-smokers). The BMI values for the total population were higher at the end of the study. There were no differences in BMI between U-cot groups.

Classification based on urinary cotinine levels was the same in both campaigns. Concerning self-reported information, the only participant who claimed to have quit smoking between 2010 and 2011 was considered to continue smoking as the U-Cot value for 2011 was high (604 μg/L). Based on the questionnaire, one participant declared to be a smoker although his U-cot level were <30 μg/L, and other as non-smoker, but had U-cot levels >100 μg/L. The number of self-reported cigarettes smoked per day the week prior to sample collection did not decrease significantly (13 ± 2 vs.11 ± 2, $n=15$) among both campaigns. No subject smoked more than 20 cigarettes daily. Self-reporting information on secondhand smoke exposure for any period of the week was answered by 79 participants, 62 with U-cot <50 μg/L.

Table 2 presents U-cot_{cr} and U-Cd_{cr} values in terms of U-cot stratification. In 2011, 60% of the population had lower U-cot_{cr} levels than in 2010 (62% and 53% in low and high U-cot groups, respectively). Only the low U-cot group (non-active smokers) showed a significant variation in the pair-wise comparison for the two sampling periods ($p<0.05$).

Based on self-reported information, the decrease in U-cot_{cr} level in the group of non-active smokers (U-cot <50 ug/L) was significant only in those which were not exposed to SHS ($p=0.012$, Fig. 1a). The cotinine levels in 2011 was significantly lower for those not SHSe at weekends ($p=0.029$), while no differed between exposed or not at working days (from Mondays to Thursdays) (Fig. 1b).

Approximately 1–8% of study participants had urinary cadmium levels (U-Cd) below the LOQ before and after introduction of the anti-smoking legislation, respectively. The highest value (one volunteer with almost 1 μg Cd/g creatinine) was obtained in 2010. In general, a decrease in U-Cd_{cr} was observed in 2011 when compared to 2010 in 76% of participants. Cadmium in 2011 was lowest for group with U-cot <50 μg/L in comparison both with its previous values ($p<0.001$) and with the U-cot ≥50 μg/L group ($p<0.05$) after introduction of stricter anti-smoking legislation (Table 2). No difference in U-Cd_{cr} were found in relation to SHSe.

Measures of correlation, variation and reliability for all analytes can be found in Table 3 (a,b and c). The proportions of total outcome variance due to inter-individual variation, i.e. including external factors, were estimated as the intra-class correlation coefficient (Table 3a). For U-Cd, the inter-variation between the two measures was 55% expressed in volume and 72% as creatinine-corrected levels, Creatinine per se showed less inter-variation (45%) than Cd, and U-cot variation was mainly external (93–96%), as expected.

In Table 3b, Spearman's coefficient was 0.8 for cadmium (U-Cd_{cr}) and 0.4–0.5 for creatinine between 2010 and 2011 ($p<0.001$), irrespective of U-cot group. The correlation between creatinine-corrected and -uncorrected Cd levels was slightly higher after the law came into force, while U-cot correlated well between both years and between its volume and creatinine-corrected values. However, U-cot ≥50 μg/L group always showed the lowest correlation value between creatinine-corrected and -uncorrected values for U-Cd and U-cot.

Table 2
Levels of urinary cotinine and cadmium, both creatinine-corrected, in the two groups stratified by cotinine levels in the two campaigns studied (2010 and 2011, $N=83$).

Group	U-cot_{cr} (μg/g)				U-Cd_{cr} (μg/g)			
	2010[a]	2011[a]	p[a]	%[c]	2010[a]	2011[a]	p[a]	%[c]
U-cot <50μg/L ($N=66$)								
Median	0.8	0.7	0.049	62	0.17	0.10	<0.001	76
p25	0.5	0.4			0.11	0.06		
p75	1.2	1.0			0.29	0.22		
U-cot ≥50μg/L ($N=17$)								
Median	839.8	865.7	NS	53	0.25	0.24	NS	76
p25	318.0	443.2			0.17	0.17		
p75	1167.2	1262.2			0.43	0.38		
pMW-test[b]	<0.001	<0.001			NS	0.014		

[a] Wilcoxon test for related samples comparing 2010 and 2011 individual values.
[b] Comparison between U-cot groups by Mann–Whitney test.
[c] Percentage of individuals who showed an analyte reduction. No significant difference between U-cot levels groups, by z-test.

Table 1
Descriptive parameters of the study population in the two sampling campaigns 2010 and 2011, before and after smoking-free law entered into force respectively. Groups are stratified by cotinine level in urine considering active smokers those with cotinine levels above or equal 50 μg/L. Volunteers were recruited in Madrid Region, Spain.

	***n* (%)**[a]	**Age** (years) 2010[b]	**BMI (kg/cm^2)**[b]			
			n	**2010**	**2011**	***p***[c]
All	83 (100)	44.5 ± 9.4 (44.0)	80	24.8 ± 4.9 (24.1)	25.3 ± 4.7 (24.1)	<0.001
Women	59 (71.1)					
Men	24 (28.9)					
U-Cot <50 μg/L	66 (79.5)	44.9 ± 9.0 (44.5)	64	24.7 ± 4.1 (24.1)	25.0 ± 4.0 (24.1)	0.001
Women	47 (71.2)					
Men	19 (28.8)					
U-Cot ≥50μg/L	17 (20.5)	42.9 ± 10.7 (39.0)	16	25.1 ± 7.4 (23.6)	26.2 ± 7.0 (24.1)	0.010
Women	12 (70.6)					
Men	5 (29.4)					
pMW-test[d]		NS		NS	NS	

[a] Arithmetic mean $\pm$ SD (median). BMI, Body Mass Index.
[b] Gender proportions between U-cot groups, z-test: NS.
[c] Wilcoxon test for related samples, comparing 2010 and 2011 individual values.
[d] Mann Whitney U-test for independent samples, comparing U-Cot groups.

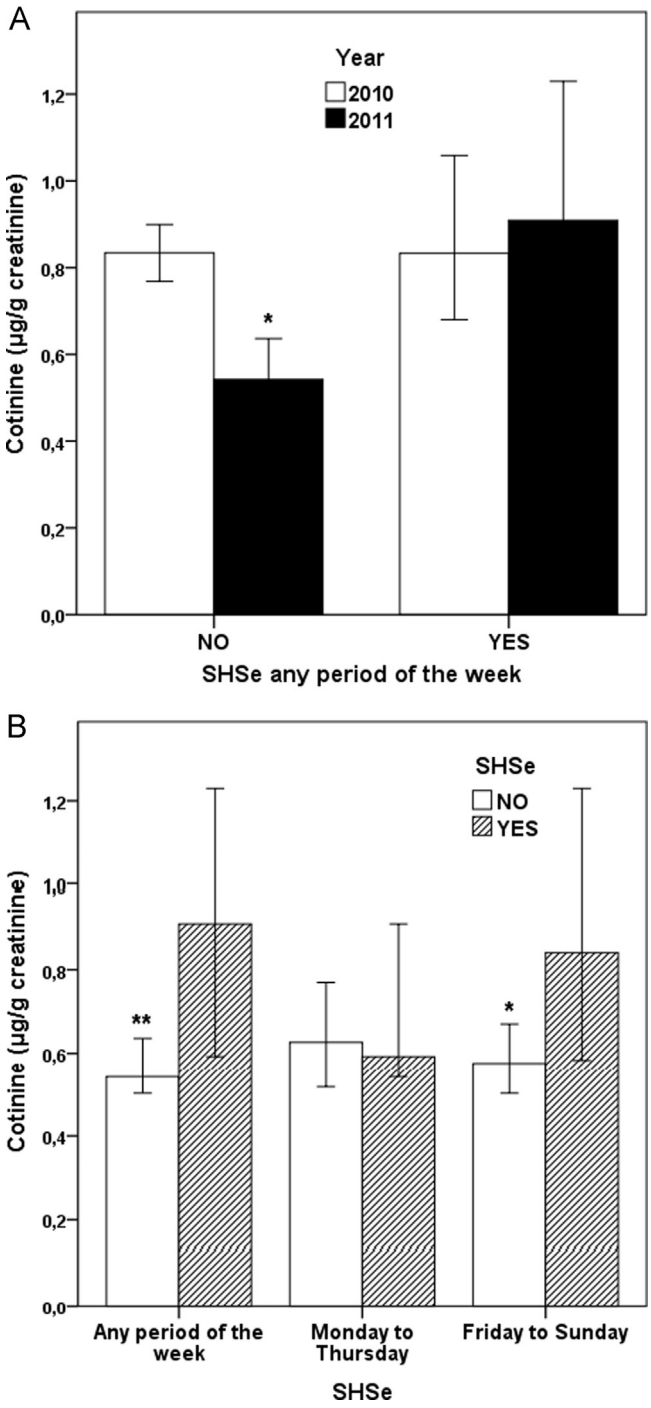

Fig. 1. Comparison of creatinine corrected cotinine levels (U-cot$_{cr}$) in the group of non-active smokers (U-cot < 50 µg/L) according to self-reported information on Second Hand Smoke exposure (SHSe). Medians and CI 75% (N=62). (A) SHSe at any period of the week: U-cot$_{cr}$ before (2010) and after (2011) application of stricter antismoking ban in Spain were compared by Wilcoxon test for related samples, $^{*}p$=0.012. (B) SHSe at different periods of the week for 2011 samples. Mann–Whitney U-test: $^{*}p$=0.029; $^{**}p$=0.009.

Both U-Cd and U-cot, creatinine-corrected or not, showed the same good correlation with the self-reported number of cigarettes smoked per day in 2010, whereas no such associations were found in 2011 for creatinine-corrected analytes (Table 3c). The good correlation obtained for active smokers in 2010 between U-Cd and U-cot, creatinine-corrected or not, decreased after the law came into force.

Table 3a
Reproducibility and correlation measures for the sampling campaigns before and after the anti-smoking legislation came into force. Intra-class correlation coefficient, biomarker Ln-transformed (N=83, p < 0.001).

	ICC (95%CI)	
	µg/L	µg/g creatinine
Cadmium (U-Cd)	0.55 (0.36–0.70)	0.72 (0.39-0.85)
Cotinine (U-cot)	0.96 (0.93–0.97)	0.93 (0.90-0.96)
Creatinine	0.45 (0.26–0.60)	–

Table 3b
Reproducibility and correlation measures for the sampling campaigns before and after the anti-smoking legislation came into force. Spearman's coefficient between concentration measures of the same analyte (N=83).

		2010 vs 2011	vs µg/L	
			2010	2011
U-Cd$_{cr}$ (µg/g creatinine)	All	0.82***	0.70***	0.83***
	U-cot < 50µg/L	0.78***	0.70***	0.83***
	U-cot ≥ 50µg/L	0.77**	0.48	0.63**
Creatinine (µg/L)	All1	0.43***	–	–
	U-cot < 50µg/L	0.53*	–	–
	U-cot ≥ 50µg/L	0.53*	–	–
U-cot$_{cr}$ (µg/g creatinine)	All1	0.65***	0.79***	0.73***
	U-cot ≥ 50µg/L	0.55*	0.57*	0.56*

* p < 0.05
** p < 0.01
*** p < 0.001

A residual negative correlation between creatinine and both U-Cd$_{cr}$ and U-cot$_{cr}$ was obtained upon creatinine correction, thereby suggesting an over-adjustment and a residual influence of diuresis on Cd levels. In 2011, the coefficient for U-Cd$_{cr}$ decreased to non-significant values when U-cot < 50 µg/L were included, i.e. Spearmans' rho for this group changed from −0.32 (p=0.01) in 2010 to −0.14 in 2011, while the correlation between U-Cd and U-Cd$_{cr}$ was higher (Table 3b). A linear fit of volume and creatinine-corrected indicators of cadmium and creatinine concentration with respect to cotinine (Fig. 2) showed that creatinine and both U-Cd concentration expressions decrease as U-cot decreases. U-Cd and U-Cd$_{cr}$ tend to converge on the same value (about 0.2) for U-cot < 500 µg/L, i.e. the creatinine value appears to be unitary (1 g/L) at these metal and cotinine concentrations.

4. Discussion

Urinary cadmium levels are assumed to be proportional to kidney Cd stores and to reflect overall body burden as long as kidney function remains intact (EFSA, 2009; Järup and Åkesson, 2009). The median U-Cd levels found in the urine of our volunteers are far lower than 1 µgCd/g creatinine, the European Food Safety Authority threshold value for protection against kidney damage, thereby suggesting the lack of an important exposure source of this metal (EFSA, 2009). However, a reduction in Cd exposure is still relevant as adverse effects have been reported for the kidney and on bone and neurodevelopmental deficits at U-Cd$_{cr}$ concentrations as low as 1 µgCd/g creatinine (Järup and Åkesson, 2009, Ciesielski et al., 2012). Even at lower concentrations (< 0.50 µg/g creatinine) Cd has been reported to be associated with hypertension and cardiovascular disease (Tellez–Plaza et al., 2012b), breast cancer incidence (McElroy et al., 2006) and mortality (Menke et al., 2009; Adams et al., 2012).

Table 3c
Reproducibility and correlation measures for the sampling campaigns before and after the anti-smoking legislation came into force. Spearman's correlation coefficient between different analytes ($N=83$).

	Cigarettes/day		U-cot (μg/L)		U-cot$_{cr}$ (μg/g creatinine)		Creatinine (g/l)	
	2010	2011	2010	2011	2010	2011	2010	2011
U-Cd (μg/L)								
All			0.20	0.27*			0.40***	0.41***
U-cot ≥ 50μg/L	0.65**	0.63*	0.80***	0.29			0.47	0.28
U-Cd$_{cr}$ (μg/g creatinine)								
All					0.27*	0.27*	−0.32**	−0.14
U-cot ≥ 50μg/L	0.57*	0.49			0.68*	0.45	−0.46	−0.50*
U-cot (μg/L)								
All							0.14	0.06
U-cot ≥ 50μg/L	0.75**	0.70**					0.42	−0.02
U-cot$_{cr}$ (μg/g creatinine)								
All							−0.41***	−0.55***
U-cot ≥ 50μg/L	0.57*	0.34					−0.43	−0.77***

** $p < 0.01$
* $p < 0.05$
*** $p < 0.001$

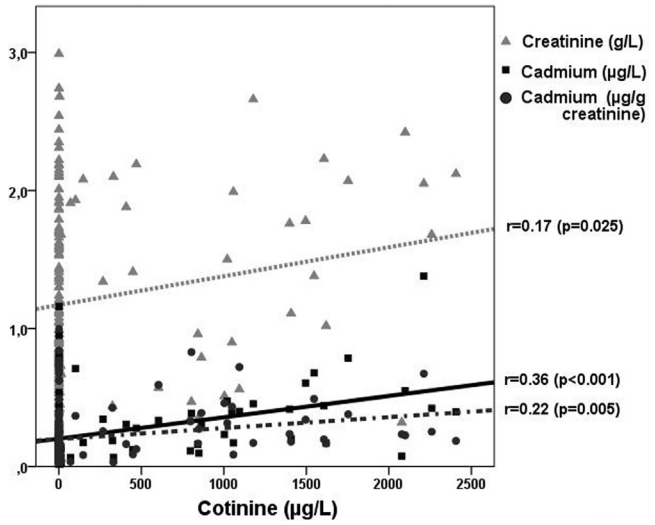

Fig. 2. Linear fit of volume (μg/L) and creatinine-corrected (μg/g) indicators of cadmium and creatinine with respect to cotinine. Creatinine levels decrease and both Cd values tend to converge as cotinine decreases to below < 500 μg/L. Pearson correlation coefficient (r) and significance level (p) were given for creatinine ($r=0.17$, $p=0.025$) and uncorrected and creatinine-corrected Cd ($r=0.36$, $p<0.001$ and r=0.22, $p=0.005$, respectively).

We found that the smoking biomarker cotinine (U-cot$_{cr}$) decreased significantly in passive smokers, i.e. those with U-cot < 50 μg/L, especially if these subjects declared not to be SHSe ($p=0{,}012$). In general, cotinine decrease mirrored in the smoking habit countrywide, since the number of cigarettes sold in 2011 decreased by 16.7% with respect to 2010 and the smoke exposure at work and in indoor public places and transport reduced dramatically respect to 2006 (INE, 2013).

Spanish indoor workplaces, bars and restaurants that became smoke-free experienced improved air quality, as measured by nicotine in air (Nebot et al., 2009) or PM2.5 concentration (Córdoba et al., 2013). Law 42/2010 was expected to reduce smoke exposure in public places mainly at weekends, since exposure during the week (Monday to Thursday) is either home or tends to be more work-related, and the last one was regulated previously (Law 28/2005). Accordingly, in 2011 we found a statistical significant difference in U-cot$_{cr}$ levels between those SHEe at weekends and not for those SHEe from Monday to Thursday. Thus, after implementation of the latest smoking ban legislation in 2010, home happened to be the most common site for smoke exposure (17.8%) (INE, 2013). Callinan et al.'s (2010) meta-analysis revealed that, despite a greater reduction in SHSe in bar and restaurant workers upon implementation of a legislative ban, the prevalence or duration of self-reported exposure at home did not change. As such, there continues to be a need to reduce smoking in enclosed areas and/or to promote smoke ventilation. Better informed smokers are more likely to have smoke-free homes and to abstain from smoking in a room with children (Evans et al., 2012).

Cadmium levels decreased significantly too in the group with U-cot < 50 μg/L but showed less variations and more marked reduction than urinary cotinine levels (Table 2). Wu et al. (1995) found that airborne Cd was the most promising environmental marker for tobacco smoke as it has a similar content and emission factor for different brands of cigarettes and is found at elevated levels in fine respirable particles. These particles can penetrate the deepest parts of the human lung and affect the cadmium body burden.

The decrease in cadmium levels (U-Cd$_{cr}$) found in 76% of our participants indicates a positive change between both years. Our results comparing individual levels showed this effect just a year after implementation of the stricter anti-smoking legislation, probably favoured by the low concentration detected and the stability of our population. However, the more significant Cd than cotinine reduction might also be favoured by the less average air Cd concentration detected in Madrid (0.6 ng/m^3 in 2010 and 0.4 ng/m^3 in 2011) (Madrid, 2011) and by the BMI increment (Riederer et al., 2013).The observed trends should be revaluated in the future years to confirm these findings in a long term. NHANES U-Cd reduction concentrations have been reported over a 20-year period (1988–2008) by Tellez-Plaza (2012a) who highlighted the decrease in smoking rates and changes in tobacco smoke exposure.

The intra-class correlation coefficient (ICC) for U-Cd values was higher than the estimates of less than 50% found by Gunier et al. (2013) and Karmaus et al. (2008). Although this might mirror changes in smoke exposure, the non-randomness of our population may lead to an overestimation of the inter-person variance and underestimate the intra-person variance, thereby resulting in a higher ICC. For the group with levels of U-cot ≥ 50 μg/L

(Table 3c), we found a higher correlation between both biomarkers, before introduction of the new law (0.68, $p<0.05$ vs 0.45, NS), thus suggesting that smoke was a more important source of Cd in 2010 than in 2011. This association was low for the population as a whole (0.3, $p<0.05$) and persisted in time, as would be expected if tobacco is not the main Cd source for the general population, particularly non-smokers (ATSDR, 2012). Nevertheless, the reduction in Cd urine concentration over the year suggests that the change was registered at both an inter- and intra-individual level.

In the data considered here, U-Cd_{cr} values obtained before implementation of the stricter anti-smoking legislation were found to be negatively correlated to creatinine, thus indicating an over-adjustment and a residual influence of diuresis on Cd levels, in agreement with Chaumont et al. (2013). This association, considering all participants in our study, was not significant after introduction of the legislation, whereas the correlation between U-Cd and U-Cd_{cr} was higher. It was remarkable that both Cd indicators tend to converge on the same value at low U-cot levels, i.e. the creatinine value tends to be unitary. It seems possible that a global reduction in environmental smoke or nicotine exposure might favor this effect, and the physiological response to these changes may form part of the "unidentified time-related factors" responsible for the unexplained Cd decreases mentioned by Riederer et al. (2013).

Smoke exposure, which can be represented by U-cot, has been associated with altered kidney structure and function (Wong et al., 1992; Orth, 2002; Dülger et al., 2011; Budisavljevic and Ploth 2012). On the other hand, renal creatinine clearance is largely a function of glomerular filtration and some tubular secretion (Jatlow et al., 2003) and nicotine's antidiuretic effect (Miller and Moses, 1976) or glomerular hyperfiltration (Orth, 2002) might mediate the higher urinary creatinine levels found in active smokers by Dülger et al. (2011) and the slight creatinine increment associated with cotinine observed in this study ($r=0.17$, $p=0.025$). As such, creatinine-corrected cadmium (U-Cd_{cr}) values are likely to underestimate body Cd burden for smokers with respect to the uncorrected U-Cd indicator and the lost relationship with cigarettes smoked.

In addition, if creatinine, Cd or diuresis in general were altered by smoke components, the U-Cd values might not reflect either kidney or body burden or the same proportion of Cd burden between smokers and non-smokers. Thus, Chaumont et al. (2013) have reported an unexpectedly low increase in U-Cd for smokers despite their continued higher exposure. As such, changes to renal function that may result from smoking, which was long regarded as merely an additional Cd source, should also be taken into consideration. On the other hand, Cd alone could alter the results as it might reduce cotinine clearance and induce the p-450 hepatic metabolizing enzyme that participates in the conversion of nicotine to cotinine (Satarug et al., 2004a).

Some limitations should be taken into account for the present study alongside other sources of variability and bias that could not be accounted for. One of these is that the limited sample sizes for certain population groups might have resulted in imprecise assessments. Another one are the imprecision of a self-administer questionnaire which finding of questions not answered which prevent some comparisons or deeper analysis. Dietary Cd intake or other sources of exposure were not expected to change over the period considered as the population studied comprised adult non-occupationally exposed workers (civil servants), who are relatively homogeneous in terms of age, ethnicity and education and with a lifestyle which tends to be stable.

To the best of our knowledge, this study is the first to evaluate the impact of smoke-free legislation on the U-cot and U-Cd values for the same subjects. One year post-implementation, we found indicators of a reduction in smoke exposure in non-active smokers and a decrease in the body burden of Cd even at the low levels registered. Although variations of 20% in U-Cd_{cr} have been described in overnight samples of urine (Akerstrom et al., 2014), the most striking finding for our population is that the Cd values decreased in 2011 with respect to those obtained in 2010 for 76% of participants, thus prompting us to search for intra-individual comparisons. This Cd decrease includes the analytical variability (around 10%) and uncontrolled media changes, but our findings nevertheless suggest the direction that future, confirmatory studies should take. Although the follow-up (longitudinal) study for several times or years after introduction of the legislation will provide higher evidences, these results highlight the short-term effectiveness of such measures on health, with likely long-term outcomes including a lower prevalence of renal disease and its associated cardiovascular morbidity. The perceived health impact of smoke-related policies might renew interest in, and reinforce, such policies and may also lead to the promotion and/or modification of new policies, such as campaigns to avoid SHSe or ventilate smoking areas in the home.

5. Conclusions

The stricter anti-smoking legislation was accompanied by a decrease in cotinine (U-cot_{cr}) in non-active smokers, which is indicative of lower second hand smoke exposure. U-Cd_{cr}, as a biomarker of metal exposure, shows a healthy reduction in the body burden for the same passive smokers studied. Home-based smoke exposure continues to be a concern, thus highlighting the need to invest in public awareness campaigns to endorse private decisions.

Creatinine-corrected cadmium (U-Cd_{cr}) values are likely to underestimate body Cd burden at least for cotinine values above 500 μg/L, when compared to the uncorrected U-Cd indicator.

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